IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: LANDEGREN=1A

In re Application of:	)	Art Unit: 1637
	)	
Ulf LANDEGREN et al	)	Examiner: S. Chunduru
	)	
Appln. No.: 09/785,657	)	Washington, D.C.
	)	
Date Filed: February 20, 2001	)	Confirmation No. 5356
	)	
For: METHODS AND KITS FOR PROXIMITY PROBING	)	March 26, 2005

DECLARATION UNDER 37 CFR §1.132

U.S. Patent and Trademark Office
2011 South Clark Place
Customer Window, **Mail Stop**
Crystal Plaza Two, Lobby, Room 1B03
Arlington, Virginia 22202

Sir:

I, Ulf LANDEGREN, hereby declare and state as follows:

I am a coinventor of the above-identified application and my educational and professional experience is presented in the curriculum vitae attached hereto.

It is my understanding that the claims of the above-identified application have been rejected under 35 U.S.C. §103(a) as being unpatentable/obvious over my own prior patent publication, WO 97/00446, as the primary reference and Ebersole et al., WO 97/32044, as the secondary reference.

The experiments described below, which demonstrate the unexpected results obtained by the liquid phase (solution) based proximity probe detection method (hereinafter referred to as the homogeneous assay or Prox II) of the present invention claimed in the

above-identified application in comparison to the solid phase anchored proximity probe detection method of WO 97/00446 (hereinafter referred to as the solid phase assay or Prox I), were conducted by me or under my direct supervision, and I can attest of my own personal knowledge that all the results reported herein are true and accurate.

A proximity probe pair consisting of SELEX-aptamer derived binding moieties with extended oligonucleotides for proximity dependent ligation was used to detect the analyte, Platelet derived growth factor BB (PDGF-BB). For the solid phase anchored assay (Prox I), the analyte PDGF-BB and the proximity probes bound thereto were first immobilized by an antibody specific for the analyte and coupled to the solid support. Excess amounts of proximity probes were then removed by washing the support. The proximity probes bound to the immobilized analyte were then ligated and the ligation product subsequently detected by real-time PCR.

In the homogeneous assay (Prox II), the proximity probes were mixed with the sample containing analyte, equilibrated, and then ligated together without the removal of excess probes or immobilization of the target analyte. The ligation product was detected and quantified by real-time PCR.

The same target antigen and proximity probe pair were used in both experiments. Both assays were conducted under conditions optimized for maximum sensitivity. Figure 1 presented below schematically illustrates the solid phase assay (Prox I) compared to the homogeneous proximity probe assay (Prox II).

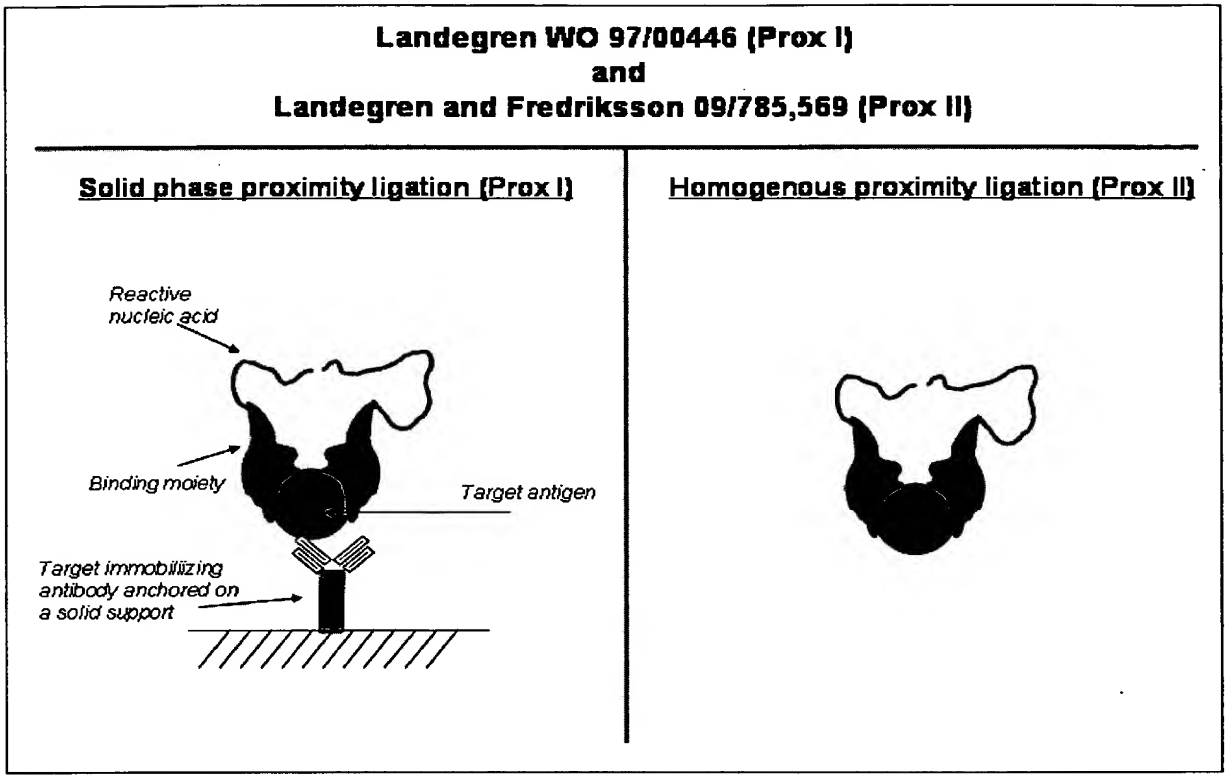

FIG. 1

The assays were performed as follows:

Solid phase anchored assay (Prox I)

Detection of PDGF-BB by solid phase proximity ligation. Five hundred nanogram of an anti-PDGF antiserum immunoglobulin fraction in 20 μl was added to optical tubes for real-time PCR, followed by blocking with 1% BSA. Up to 200 μl of sample was then incubated together with 5 nM of proximity probes A1 and A2. Unbound probes were removed by washes using a multichannel pipette (8 times with 3 flushes of 200 μl PBS 0.02% SDS, PBS and finally H_2O). A ligation mix (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.15 mM ATP, 50 nM connector oligonucleotide, 2 U T4 DNA ligase in 20 μl) was added and the

reactions were kept at 30°C for 5 minutes followed by 20 minutes at 80°C. Next, a PCR mix was added to a final volume of 50 μl, containing ROX internal fluorescence standard, 0.1 mM dNTPs, 0.2 μM primers (forward: 5'-atgtggtctat gtcgtcgttcg-3', reverse: 5'-tgagtaagaacagcgcg cat-3'), 50 nM probe for the 5' nuclease assay, 1 unit AmpliTaq Gold polymerase. After a five minute ligation reaction at room temperature, the reactions were transferred to the real-time PCR instrument for temperature cycling; 95°C for 10 minutes and then 95°C 15 seconds and 60°C 60 seconds, repeated 45 times (ABI PRISM 7700). The number of ligation products was calculated from a standard curve of diluted amplicons.

Homogeneous liquid phase assay (Prox II)

One μl aliquots, containing varied amounts of PDGF-BB diluted in 137 mM NaCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.4, 2.7 mM KCl, 1 mM $MgCl_2$, 1% bovine serum albumin (BSA), were added to optical tubes (Applied Biosystems, Foster City, CA) containing 20 pM of the proximity probes A1 and A2 in a total volume of five μl 50 mM KCl, 10 mM Tris-HCl pH 8.3, 3.3 mM $MgCl_2$, and 0.1% BSA. Upon addition of the combined mix for ligation and amplification the samples contained 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.4 units T4 DNA ligase, 400 nM connector oligonucleotide, 80 μM ATP, ROX internal fluorescence standard, 0.2 mM dNTPs, 0.5 μM primers, 50 nM probe for the 5' nuclease assay, and 1.5 units AmpliTaq Gold polymerase (ABI) in 50 μl. After a five minute ligation reaction at room temperature, the reactions were transferred to the real-time PCR instrument for temperature cycling; 95°C for 10 minutes and then 95°C 15 seconds and 60°C 60 seconds,

repeated 45 times (ABI PRISM 7700). The number of ligation products was calculated from a standard curve of diluted amplicons.

Results

As can be seen in Figure 2 below, which is a comparison between the solid phase anchored and the homogeneous proximity ligation assays for the detection of PDGF-BB as analyte, the homogeneous proximity ligation assay (Prox I) can detect about 10 times lower amounts of the target analyte than the solid phase anchored version of the assay. Signal to noise ratios on the Y-axis denote the number of ligation reactions derived from the reactions with target analyte present (signal) divided by the number of ligations derived from a reaction with no target present (noise). Furthermore, not only is the homogeneous proximity ligation assay of the present invention (Prox I) a simpler assay to perform than the solid phase anchored assay (Prox II) since it requires no washing steps, but it also consumes over 10,000 times less of the probes, $100x10^{-18}$ moles (attomoles) compared to $1x10^{-12}$ moles (picomoles).

Since the homogeneous proximity ligation assay contains no washing steps, it was initially expected that the homogeneous proximity ligation assay would produce too many background ligation events, far surpassing the signals from target binding events, and therefore would result in an insensitive assay. Surprisingly, however, the background in the homogeneous ligation assay was discovered to be very low since the reactivity of the nucleic acids at a low concentration in solution was found to be low in comparison to those bound to the target analyte. Target analyte bound proximity probes gain greatly in reactivity due to

the enhanced effective concentration of the reactive nucleic acids in the local immediate environment surrounding the analyte.

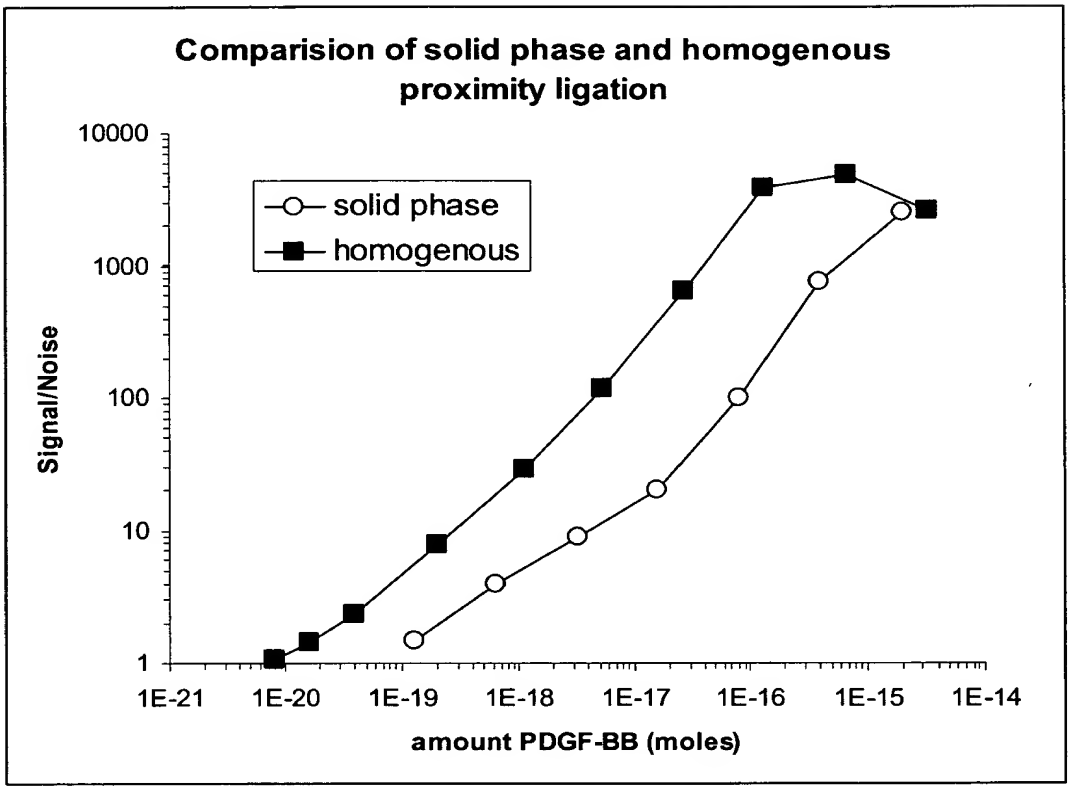

Fig. 2

In conclusion, the inventors of the present above-identified application have discovered unexpectedly superior results for the homogeneous proximity ligation assay (Prox II) over what was obtained with the solid phase proximity ligation assay (Prox I) of WO 97/00446. The unexpectedly superior results demonstrate that the homogeneous proximity ligation assay, compared to the solid phase proximity ligation assay, has a significantly lower limit of detection for the analyte while requiring much less amounts (by many orders of magnitude)

of proximity probes, thereby lowering reagent consumption, as well as being easier and more rapid to perform because it omits at least the extra steps of immobilizing antibodies/analyte to a solid support and of washing to remove unbound proximity probes found in the solid phase assay.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

March 26, 2005
Date

Ulf LANDEGREN

G:\SN\B\Bran\LandegrenUA\PTO\declaration 1.132.doc

CURRICULUM VITAE

ULF LANDEGREN
Date and place of birth: June 8, 1952, Sweden
Marital Status: Married. Three children, born 1984, 1986, and 1987
Citizenship: Swedish

OFFICE
Department of Genetics and Pathology, Rudbeck Laboratory, Se-75185 Uppsala, Sweden
Visit: Dag Hammarskjölds väg 20
Tel: +46 (0)18 4714910; Fax: +46 (0)18 471 4808. Mobile: +46 (0)708 962604
E-mail: ulf.landegren@genpat.uu.se Web: http://www.genpat.uu.se/molme/molme.html

EDUCATION

1984-1988 Postdoctoral studies in Molecular Biology at California Institute of Technology. Supervisor: Dr. Leroy Hood
1979-1984 Graduate studies in Cellular Immunology at Uppsala Biomedical Center, Sweden. Advisor: Dr. Hans Wigzell. PhD 1984.
1973-1979 School of Medicine, University of Uppsala. MD 1979.
1972-1973 Mathematics, one year. University of Uppsala, 1972

PROFESSIONAL HISTORY

1996- Professor of Molecular Medicine at Uppsala University
1993-1996 Associate Professor of Medical Genetics
1991-1993 Assistant Professor of Medical Genetics
1989-1991 Scientist at the Department of Medical Genetics, Biomedical Center of Uppsala, Sweden
1988-1989 Coordinator of the DNA Diagnostics Program, Division of Biology, California Institute of Technology (Caltech), Pasadena, California
1984-1988 Research Fellow, Division of Biology, Caltech
1979-1984 Graduate Student and Teaching Assistant, Department of Immunology, Biomedical Center of Uppsala, Sweden
1977-1984 Assistant Medical Doctor at Departments of Medicine, Surgery, and Gynecology for a total of around one and a half years
1971-1972 Laboratory Assistant, Department of Genetics and Plant Breeding, Agricultural College of Sweden, Uppsala

TEACHING

1996-2002 Member of the committee for education at the medical faculty of Uppsala.
1989-1995 Coordinator of the Medical Genetics courses at the School of Medicine, University of Uppsala
1992-present Organizer and coorganizer of six advanced international theoretical and practical courses in molecular medicine, and five corresponding courses for clinicians
1979 - 1984 Lectures and supervision of lab work during courses in Immunology and Medical Immunology at Uppsala University

COMMISSIONS OF TRUST

2004 – present Founder, board member, and chairman of the scientific board of Olink AB, Uppsala Sweden
2004 – present Member of the scientific advisory board of the Max Planck Institute for Molecular Genetics, Berlin, Germany
2004 – present Member of the board of the Center for Science and Technology Studies, Uppsala University
2004 – present Visting senior scientist at the lab of Dr Yoshihide Hayashizaki, the RIKEN Institute, Yokohama, Japan

2003 – present Coordinator of the EU Frameworkprogram 6 Integrated Project "MolTools" (www.moltools.org)

2003 – 2004 Chair of the Life Science Technology committee of the Swedish Strategic Foundation committee for Future Research Leader awards

2003 - present Member, Swedish Research Council committee on EU Research in Life Science, Genomics and Biotechnology for Health

2002 – present Coordinator of biotechnology at Uppsala University

2001 – present Cofounder and chairman of the scientific advisory board of Pal Allele BioScience, South San Francisco

2001 – present Member of the Royal Society of Sciences, Sweden

1999 - 2001 Director of the Uppsala node of the Wallenberg Consortium North for Functional Genomics

1999 – present Swedish representative in a network for functional genomics, supported by the European Science Foundation

1998 - present Member of the scientific advisory board of Sequenom, San Diego, CA, USA

1998 – present Member of the Human Genome Organization (HUGO) committee on intellectual property rights

1998 - 1999 Coorganizer of a series of international meetings on single-nucleotide polymorphisms and complex genome analysis

1997 - 2001 Member of the board of directors of Professional Genetics Laboratory AB

1995 - 1999 Coorganizer of a semiannual series of international meetings on Mutation Detection

1995 - 2003 Member of the Swedish Research Council for Medicine, Review committee on Cell Biology I

1993 – 1996 Consultant in Molecular Biology for Pharmacia Biotech, exploratory research

1993 - 2001 Consultant in Molecular Medicine at the Department of Medicine, Uppsala Academic Hospital

1991 - present Member of the Human Genome Organization (HUGO)

Member of the board of editors of **Genome Research,** published by Cold Spring Harbor Laboratory Press until 2004, **Human Mutation,** Published by John Wiley and sons, **Mutation Research Genomics,** published by Elsevier, **Compative and Functional Genomics,** Published by Wiley-VCH, and **er-Molecular Medicine** - an on-line journal

Reviewer of manuscripts also for **Science, Nature Genetics, Nature Biotechnology, Nature Medicine, Nucleic Acids Research, Proc Natl Acad Sci USA, Clin Chem, Biotechniques, Human Genetics,** etc

Member of committees evaluating research in Germany. Reviewer of grant applications for the Swedish Research Council for Medicine, the Swedish Strategic Foundation, the EU, Wellcome Foundation, US Department of Agriculture, and the research councils of Italy, Israel, The Netherlands, Norway etc

STIPENDS

The Eric K. Fernström award in 1993
The Bertil Åberg Biotechnology award in 1993
The Procordia Foundation, twice
The Nilsson-Ehle Foundation, twice
The Borgströms Foundation, four times
The Svensson Foundation
The Lennander Foundation
Fellowship from the Knut och Alice Wallenberg Foundation for postdoctoral research (Declined an EMBO postdoctoral fellowship)

FORMER GRADUATE STUDENTS (year of graduation and present position)

Maria Lagerström Fermér (PhD 1995), Assistant professor, Associate director, Head of Genetics, Molecular Sciences, AstraZeneca R&D Mölndal, Sweden

Martina Samiotaki (PhD 1996), Senior Research Officer, Alexander Research Institute, Athens, Greece. www.fleming.gr

Mats Nilsson (PhD 1998), Assistant professor, dept of genetics and pathology, Uppsala

Charlotta Olsson (PhD 2001) Sales representative, BioRad Sweden

Dan-Oscar Antson (PhD 2001), postdoc, Public Health Research Institute of New York, USA

Anette Hagberg (PhD 2002), postdoc, Swedish University of Agricultural Sciences, Uppsala

Simon Fredriksson (PhD 2002), postdoc, Stanford, USA

Martina Mendel-Hartvig (PhD 2002), Scientist, General Electric Healthcare, Uppsala

Mats Gullberg (PhD 2003), postdoc dept of genetics and pathology, currently paternity leave

Johan Banér (PhD 2003) postdoc, Swedish University of Agricultural Sciences, Uppsala

POSTDOCTORAL STUDENTS

Marek Kwiatkowski
Keith Shappert
Harvest Feng Gu
Alexander Khorlin
Mats Nilsson
Volker Gurtler
Ola Söderberg
Simon Ferdriksson
Mats Gullberg
Johan Banér
Masood Kamali
Mathias Howell

PUBLICATIONS, Research

1. Almgård G, and Landegren U. Isoenzymatic variation used for the identification of barley cultivars. *Zeitschrift für Pflanzenzüchtung* **72**: 63-73 (1974)
2. Landegren U, Ramstedt U, Axberg I, Örn A, and Wigzell H. Cyclosporin A permits the distinction between specific and NK activity generated in a human MLC. *International Journal of Cancer* **28**: 725-730 (1981)
3. Landegren U, Ramstedt U, Axberg I, Ullberg M, Jondal M, and Wigzell H. Selective inhibition of human T cell cytotoxicity at levels of target recognition or initiation of lysis by monoclonal OKT3 and Leu2a antibodies. *Journal of Experimental Medicine* **155**: 1579-1584 (1982)
4. Landegren U, Andersson J, and Wigzell H. Mechanism of T lymphocyte activation by OKT3 antibodies. A general model for T cell induction. *European Journal of Immunology*. **14**: 325-328 (1984)
5. Landegren U. Measurement of cell numbers using an endogenous enzyme, hexosaminidase. Applications for the detection of lymphokines and cell surface antigens. *Journal of Immunological Methods* **67**: 379-388 (1984)
6. Landegren U, Andersson J, and Wigzell H. Analysis of human T lymphocyte activation in a T cell tumor model system. *European Journal of Immunology* **15**: 308-311 (1985)
7. Landegren U, and Wigzell H. Cyclosporin A inhibits a discrete step in T lymphocyte activation. *Scandinavian Journal of Immunology* **22**: 279-284 (1985)
8. Landegren U, Kaiser R, Sanders J, and Hood L. A ligase-mediated gene detection technique. *Science* **241**: 1077-1080 (1988)
9. Levedakou EN, Landegren U, and Hood L. A strategy to study gene polymorphisms by direct sequence analysis of cosmid clones and amplified genomic DNA. *Biotechniques* 7: 438-442 (1989)
10. Nickerson D, Kaiser R, Lappin S, Stewart J, Hood L, and Landegren U. Automated DNA diagnostics using an ELISA-based oligonucleotide ligation assay. *Proceedings of the National Academy of Sciences USA* **87**: 8923-8927(1990)
11. Lagerström M, Dahl N, Nakahori Y, Bäckman B, Landegren U, and Petterson U. A deletion in the amelogenin gene in a family with X-linked amelogeneisis impefecta. *Genomics* **10**: 971-975 (1991)
12. Lagerström M, Parik J, Malmgren H, Stewart J, Pettersson U, and Landegren U. Capture PCR: efficient amplification of DNA fragments adjacent to a known sequence in human and YAC DNA. *PCR Methods and Applications* **1**(2): 111-119 (1991)
13. Parik J, Kwiatkowski M, Lagerkvist A, Samiotaki M, Lagerström M, Stewart J, Glad G, Mendel-Hartvig M, and Landegren U. A manifold support for molecular genetic reactions. *Analytical Biochemistry* **211**: 144-150 (1993)
14. Palmieri G, Romano G, Casamassimi A, D'Urso M, Little RD, Abidi FE, Schlessinger D, Lagerström M, Malmgren H, Steen-Bondeson M-L, Pettersson U, and Landegren U. 1.5 Mb YAC contig in Xq28 formatted with sequence-tagged sites and including a region unstable in the clones. *Genomics* **16**: 586-592 (1993)
15. Lagerström M, Pettersson U, and Landegren U. Molecular basis and consequences of a mutation in the amelogenin gene, analyzed by Capture PCR. *Genomics* **17**: 89-92 (1993)
16. Kwiatkowski M, Parik J, and Landegren U. A high-capacity manifold support for the detection of specific IgE in allergic individuals. *Journal of Immunological Methods* **168**: 137-143 (1994)

17. Lagerkvist A, Stewart J, Lagerström M, Parik J, and Landegren U. Manifold sequencing: rapid processing of large sets of sequencing reactions. *Proceedings of the National Academy of Sciences USA* **91**: 2245-2249 (1994)

18. Samiotaki M, Kwiatkowski M, Parik J, and Landegren U. Dual-color detection of DNA sequence variants through ligase-mediated analysis. *Genomics* **20**: 238-242 (1994)

19. Kwiatkowski M, Samiotaki M, Hurskainen U, Landegren U. Solid-phase synthesis of chelate-labelled oligonucleotides: application in triple-color ligase-mediated gene analysis. (1994) *Nucleic Acids Research* **22**: 2604-2611 (1994)

20. Nilsson M, Malmgren H, Samiotaki M, Kwiatkowski M, Chaudhary B, and Landegren U. Padlock probes: circularizing oligonucleotides for localized DNA detection. *Science* **265**: 2085-2088 (1994)

21. Lagerström-Fermér M, Nilsson M, Bäckman B, Salido E, Shapiro L, Pettersson U, and Landegren U. Amelogenin signal peptide mutation: correlation between mutations in the amelogenin gene and manifestations of X-linked amelogenesis imperfecta. *Genomics* **26**: 159-162 (1994)

22. Sroga GE, Landegren U, Bergman B, and Lagerström-Fermér M. Isolation of *nifH* and a part of *nifD* by modified capture PCR from a natural population of the marine cyanobacterium *Trichodesmium tsp. FEMS Microbiol Lett* **136**: 137-145 (1996)

23. Tyagi S, Landegren U, Mounssef T, Lizardi P, Kramer F. Extremely sensitive diagnostic assay based om the exponential amplification of binary hybridization probes by Q_b replicase *Proceedings of the National Academy of Sciences USA* **93**: 5395-5400 (1996)

24. Lagerström-Fermér M, Sundvall M, Johnsen E, Warne, GL, Forrest SM, Zajac JD, Ravine D, Landegren U, and Pettersson U. Mapping of X-linked recessive panhypopituitarism to Xq25-q26. *American Journal of Human Genetics* **60**: 910-916 (1997)

25. Waldenström E, Lagerkvist A, Dahlman T, Westermark K, Landegren U. Efficient detection of mutations in Wilson's disease by manifold sequencing. *Genomics* **37**: 303-309 (1996)

26. Kwiatkowski M, Nilsson M, Landegren U. Synthesis of full-length oligonucleotides: cleavage of apurinic molecules on a novel support. *Nucleic Acids Research* **24**: 4632-4638 (1996)

27. Kwiatkowski M, Nilsson M, Landegren U. A support for synthesis of full-length oligonucleotides. *Collect Czech Chem Commun* **61**: S307-S310 (1996)

28. Nilsson M, Krejçi K, Koch J, Kwiatkowski M, Gustavsson P and Landegren U. Padlock probes reveal the *in situ* distribution of alpha-satellite sequences on chromosomes 13 and 21, differing in a single nucleotide position. *Nature Genetics* **16**: 252-255 (1997)

29. Samiotaki M, Kwiatkowski M, Ylitalo N, Landegren U. Seven-color time-resolved fluorescence hybridization analysis of human papilloma virus types. *Analytical Biochem*istry **253**: 156-161(1997)

30. Gu HF, Guan X-y, Zhang H, Landegren U, Bu Z, Chen W, Trent JM. Porcine chromosome painting using specific microdissected chromosome probes. *Cytogenetics and Cellular Genetetics* (1998)

31. Olsson C, Zethelius B, Lagerström-Fermér M, Asplund J, Berne C, Landegren U. The level of heteroplasmy for the mitochondrial mutation A3243G correlates with age at onset of diabetes and deafness. *Human Mutation* **12**: 52-58 (1998)

32. Banér J, Nilsson M, Mendel-Hartvig M, and Landegren U. Signal amplification of padlock probes by rolling circle replication. *Nucleic Acids Research* **26**: 5073-5078 (1998)

33. Kwiatkowski M, Fredriksson S, Isaksson A, Nilsson M, and Landegren U. Inversion of *in situ* synthesized oligonucleotides: improved reagents for hybridization and primer extension in DNA microarrays. *Nucleic Acids Research* **27**: 4710-4714 (1999)

34. Olsson C, Waldenström E, Westermark K, Landegren U, Syvänen A-C. Rapid determination of the frequencies of ten alleles in Wilson's disease gene (ATP7B), in pooled DNA samples *European Journal of Human Genetics* **8**: 933-939 (2000)

35. Barbany G, Hagberg A, Olsson-Strömberg U, Simonsson B, Syvänen A-C Landegren U. Manifold-assisted reverse transcription-PCR with real-time detection for measurement of the BCR-ABL fusion transcript in chronic myeloid leukemia patients. *Clinical Chemistry* **46**:913-20. (2000)

36. Hagberg A, Barbany G, Samiotaki M, Landegren U. Expression profiling across many samples via manifold-assisted mRNA processing. *Nucleic Acids Research* **28**:E54 (2000)

37. Nilsson M, Barbany G, Antson D-O, Gertow K, Landegren U. Enhanced RNA detection and distinction by enzymatic probe ligation. *Nature Biotechnology* **18**:791-793 (2000)

38. Antson D-O, Isaksson A, Landegren U, Nilsson M. PCR-generated padlock probes detect single-nucleotide variation in genomic DNA. *Nucleic Acids Research* **28**:E58 (2000)

39. Nilsson M, Antson DO, Barbany G, and Landegren U. RNA-templated DNA ligation as a mechanism for transcript analysis, *Nucleic Acids Research* **29**: 578-581 (2001)

40. Lagerström-Fermér M, Larhammar D, Johnsen E, and Landegren U. Comparative genomics by Capture PCR. *Genomics* **79**: 442-447 (2002)

41. Jobs M, Fredriksson S, Brookes AJ and, Landegren U. Effect of oligonucleotide truncation on single-nucleotide distinction by solid-phase hybridization *Analytical Chemistry* **1**: 199-202 (2002)

42. Fredriksson S, Gullberg M, Jarvius J, Olsson C, Pietras K, Östman A, Landegren U. Sensitive protein detection via proximity-dependent DNA ligation assays. *Nature Biotechnology* **20**: 473-477 (2002)

43. Krook H, Hagberg A, Song Z, Landegren U, Wennberg L, Korsgren L. A distinct Th1 response precedes the described Th2 response in islet xenograft rejection. *Diabetes* **51**: 79-86 (2002)

44. Antson DO, Mendel-Hartvig M, Landegren U Nilsson M. PCR-generated padlock probes distinguish homologous chromosomes through quantitative fluorescence analysis. *European Journal of Human Genetics* **11**: 357-363. (2003)

45. Hardenbol P, Banér J, Jain M, Nilsson M, Namsaraev EA, Karlin-Neumann GA, Fakhrai-Rad H, Ronaghi M, Willis T, Landegren U, Davis RW. Highly parallel genotyping with sequence-tagged molecular inversion probes and DNA microarrays. *Nature Biotechnology* **6**: 673-678 (2003)

46. Hagberg A, Barbany G, Landegren U, Birgegård G. Beta globin mRNA increases rapidly during erythropoietin treatment. *Scandinavian Journal of Clinical Laboratory Investigations* **63**: 239-245 (2003)

47. Banér J, Isaksson A, Waldenström E, Jarvius J, Landegren U, Nilsson M. Parallel gene analysis with allele-specific padlock probes and tag microarrays. *Nucleic Acids Research* **31**: e103 (2003)

48. Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, Hellstrom M, Backstrom G, Fredriksson S, Landegren U, Nystrom HC, Bergstrom G, Dejana E, Ostman A, Lindahl P, Betsholtz C. Endothelial PDGF-B retention is required for proper

investment of pericytes in the microvessel wall. *Genes and Development* **17**: 1835-1840 (2003)

49. Mendel-Hartvig, M, Kumar A, Landegren U. Ligase-mediated construction of branched DNA: A novel DNA joining activity catalyzed by T4 DNA ligase. *Nucleic Acids Research* **32**: e2 (2004)

50. Dahl F, Mendel-Hartvig M, Banér J, Gullberg M, Landegren U, Nilsson M. Circle-to-circle amplification for precise and sensitive DNA analysis. *Proceedings of the National Academy of Sciences USA* **101**: 4548-4553 (2004)

51. Gullberg M, Gústafsdóttir SM, Schallmeiner E, Jarvius J, Bjarnegård M, Betsholtz C, Landegren U, Fredriksson S. Cytokine detection by antibody-based proximity ligation. *Proceedings of the National Academy of Sciences USA* **101**: 8420-8424 (2004)

52. Wedrén S, Lovmar L, Humphreys K, Magnusson C, Melhus H, Syvanen AC, Kindmark A, Landegren U, Lagerström Fermér M, Stiger F, Persson I, Baron J, Weiderpass E. Estrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: A case control study. *Breast Cancer Research* **6**: R437-449 (2004)

53. Larsson C, Koch J, Nygren A, Janssen G, Raap AT, Landegren U, Nilsson M. Target-primed rolling-circle amplification of padlock probes for single-molecule genotyping in situ. *Nature Methods*, **1**. 227-232 (2004)

54. Banér J, Marits P, Dahl F, Nilsson M, Winqvist O, Landegren U. Parallel analysis of T cell receptor Vbeta gene expression using padlock probes and DNA microarrays. *Clinical Chemistry*, **51**: 768-775 (2005)

55. Stenberg J, Dahl F, Landegren U, Nilsson M. PieceMaker: a software tool for selector application design. *Nucleic Acids Research*, in press

56. Dahl F, Gullberg M, Stenberg J, Landegren U, and Nilsson M. Multiplex amplification enabled by selective circularization of large sets of genomic DNA fragments. *Nucleic Acids Research*, in press

57. Szemes M, Bonants P, de Weerdt M, Banér J, Landegren U, Schoen C. Diagnostic applicaiton of padlock probes – multiplex detection of plant pathogens using universal microarrays. Submitted

58. Melin J, Johansson H, Söderberg O, Nikolajeff F, Nilsson M, Landegren U, Jarvius J. A disposable microfluidic platform enabling actuatation, cell culture, and sensitive fluorescence detection. Submitted

59. Jarvius J and Landegren U. DNA Skyline: a font to view DNA sequences. Submitted

60. Stenberg J, Nilsson M, Landegren U. ProbeMaker: a framework for construction of oligonucleotide sets. Submitted

61. Söderberg O, Gullberg M, Emilson M, Bahram F, Larsson LG, Landegren U. Localized detection of proteins *in situ* using proximity ligation. In preparation

REVIEWS AND BOOK CHAPTERS

1. Landegren U, Kaiser R, Caskey CT, and Hood L. DNA diagnostics - molecular techniques and automation. *Science* **242**: 229-237 (1988)

2. Landegren U, Kaiser R, and Hood L. Oligonucleotide ligation assay. In "*PCR Protocols: A guide to methods and applications*" Ed. Innis. Academic Press. pp. 92-98 (1990)

3. Landegren U. Automated gene detection using the oligonucleotide ligation assay *in "Protocols in Human Molecular Genetics", volume 9 in the series "Methods in*

Molecular Biology", Ed. C Mathews, The Humana Press Inc., Clifton NJ. 85-93 (1991)

4. Landegren U. DNA probes and automation. *Current Opinion in Biotechology* **3**:12-17 (1992)
5. Landegren U. Detection of mutations in human DNA. *Genetic Analysis Techniques and Applications* **9**(1):3-8 (1992)
6. Landegren U. Molecular mechanics of nucleic acid sequence amplification. *Trends in Genetics* **9**(6):199-204 (1993)
7. Landegren U. Ligation-based DNA diagnostics. *BioEssays* **15**:761-765 (1993)
8. Syvänen AC and Landegren U. Detection of point mutations by solid phase methods. *Human Mutation* **3**: 172-179 (1994)
9. Lagerström-Fermér M and Landegren U. Understanding enamel formation from mutations causing amelogenesis imperfecta. *Connective Tissue Research* **32**: 241-246 (1994)
10. Cotton D and Landegren U. Interest rising in mutation detection. *Genome Digest* **2**:1-4 (1995)
11. Forrest S, Cotton R, Landegren U, and Southern E. How to find all those mutations. *Nature Genetics* **10**: 375-376 (1995)
12. Landegren U, Samiotaki M, and Kwiatkowski M. Ligase-mediated gene detection *in "Encyclopedia of molecular biology: fundamentals and applications"*. Ed. R A Meyers, VCH Publishers, Weinheim **3**: 391-399. (1995)
13. Landegren U, Samiotaki M, Nilsson M, Malmgren H, and Kwiatkowski M. Detecting genes with ligases. *Methods: a companion to Methods in Enzymology* **9**: 84-90 (1996)
14. Landegren U. Editor. Laboratory protocols for mutation detection. Oxford University Press, Oxford. 192pp ISBN 0-19-857795-8 (1996)
15. Landegren U. Mutation detection now and later. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 2-4 (1996)
16. Samiotaki M, Kwiatkowski M and Landegren U. OLA - dual color oligonucleotide ligation assay. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 96-100 (1996)
17. Lagerkvist A and Landegren U. Manifold sequencing. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 119-123 (1996)
18. Nilsson M and Landegren U. Padlock probes for in situ detection. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 135-138 (1996)
19. Parik J, Lagerkvist A, Kwiatkowski M. and Landegren U. Construction of manifold supports. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 169-172 (1996)
20. Lagerström-Fermér M and Landegren U. Capture PCR - amplification with single-sided specificity across mutation breakpoints. In "Laboratory protocols for mutation detection" Ed. U Landegren. Oxford University Press, Oxford. ISBN 0-19-857795-8 183-188 (1996)
21. Landegren U. The challengers to PCR: a proliferation of chain reactions. (Invited commentary). *Current Opinon in Biotechnology* **7**: 95-97 (1996)
22. Landegren U. Molecular analysis of genomes and genes. Elsevier. Wennergren Symposium (1996)

23. Waldenström E, Landegren U, Loof L, Westermark K. Genetisk diagnostik vid fulminant Wilsons sjukdom. *Hygiea* **105**: 177 (1966)

24. Landegren U. Reading genes that spell health and disease. (Meeting report) *Trends in Biotechnology* **14**: 329-331 (1996)

25. Cotton D and Landegren U. Going to the roots of mutation detection. *Genome Digest* **4**:12-14 (1997)

26. Landegren U and Nilsson M. Locked on target: Strategies for future gene detection. *Annals of Medicine* **29**: 585-590 (1997)

27. Landegren, U., Samiotaki, M. Kwiatkowski, M. Nilsson, M. Hagberg, A. and Gisela Barbany. DNA detection and sequence distinction through oligonucleotide ligation. In "Mutation Detection. A practical approach". Eds. Richard Cotton, Edvard Elkins, and Susan Forrest. Oxford University Press 131-139 (1998)

28. Landegren U, Nilsson M, Kwok P-Y. Reading bits of genetic information: Methods for single-nucleotide polymorphisms analysis. *Genome Research.* **8**: 769-776 (1998)

29. Barbany G, Hagberg A, Waldenström E, Landegren .U. Molecular genetic applications of streptavidin-coated manifold supports. *Biomolecular Engineering* **16**: 105-111 (1999)

30. Landegren U. A prelude to the stars: genomics in Cannes. *Trends in Biotechnology* **17**: 1-3 (1999)

31. Isaksson A, Landegren U. Accessing genomic information: alternatives to PCR. *Current Opinion in Biotechnology* **10**: 11-15 (1999)

32. Landegren U. Consulting the source code: Prospects for gene-based medical diagnostics. *Journal of Internal Medicine* **248**: 271-276 (2000)

33. Banér J, Nilsson M, Isaksson A, Mendel-Hartvig M, Antson DO, Landegren U. More keys to padlock probes: mechanisms for high throughput nucleic acid analysis. *Current Opinion in Biotechnology* **12**, 11-15, (2001)

34. Nilsson M, Landegren U Antson DO In Robinson, J. P. e. a. (ed.), *Current Protocols in Cytometry*. Supplement 16 ed. John Wiley & Sons, Inc., New York, Vol. 1, pp. 8.8.1-8.8.12 (2001)

35. Nilsson M, Landegren U Antson DO *Current Protocols in Human Genetics.* Supplement 34 ed. John Wiley & Sons, Inc., New York, Vol. 1, pp. 4.11.11-14.11.12 (2002)

36. Nilsson M, Banér J, Mendel-Hartvig M, Dahl F, Antson, DO, Gullberg M, Landegren U. Making ends meet in genetic analysis using padlock probes. *Human Mutation* **19**: 410-415 (2002)

37. Jonsson L and Landegren U. Storing and using biobanks for research. In "Ethics in biomedicine. Ed. Mats G Hansson. (2002)

38. Gullberg M, Fredriksson, Taussig M, Jarvius J, Gustafsdottir S, Landegren U. A sense of closeness: Protein detection by proximity ligation. *Current Opinion in Biotechnology* **14**: 82-86 (2003)

39. Jarvius, J., Nilsson, M. and Landegren, U. Oligonucleotide ligation assay. *Methods in Molecular Biology* **212**, 215-228 (2003)

40. Taussig M, Landegren U. Progress in antibody arrays. *Targets* **2**: 169-176 (2003)

41. Landegren U, Dahl F, Nilsson M, Fredriksson S, Banér J, Gullberg M, Jarvius J, Gustafsdottir S, Söderberg O, Ericsson O, Stenberg J, Schallmeiner E. Padlock and proximity probes for *in situ* and array-based analyses. Tools for the postgenomic era. *Comparative and Functional Genomics* **4**: 5525-530 (2003)

42. Landegren U, Schallmeiner E, Nilsson M, Fredriksson S, Banér J, Gullberg M, Jarvius J, Gustafsdottir S, Dahl F, Söderberg O, Ericsson O, Stenberg J. Molecular tools for a molecular medicine: Analyzing genes, transcripts, and proteins using padlock and proximity probes. *Journal of Molecular Recognition*, **17**: 194-197 (2004)

43. Landegren U, Nilsson M, Gullberg M, Söderberg O, Jarvius M, Larsson C, Jarvius J. Prospects for *in situ* analyses of individual and complexes of DNA, RNA, and protein molecules with padlock and proximity probes. Methods in Cell Biology Vol 75, Cytometry, IVth Edition: New Developments, Eds. Darzynkievicz Z, Roederer M, Tanke HJ. Elsevier, 787-795

44. Stenberg J, Nilsson M, and Landegren U. Ligase-mediated molecular analyses. *In "Encyclopedia of molecular biology:Fundamentals and applications"*. Ed. R A Meyers, VCH Publishers, Weinheim, 179-193

45. Gustafsdottir SM, Schallmeiner E, Fredriksson S, Gullberg M, Söderberg O, Jarvius M, Jarvius J, Howell M, Landegren U. Proximity ligation assays for sensitive and specific protein analyses. Solicited review for *Analytical Biochemistry*, in press, April 2005

MISCELLANEOUS:

1. Landegren U. Regulation of T lymphocyte activity. Studies using monoclonal antibodies and Cyclosporin A. *Thesis*, Uppsala University (1984)

2. Landegren U, Siu G, and Hood L. Development of T lymphocytes. In: *Molecular Approaches to Developmental Biology* pp. 439-452, AR Liss Inc. (1987)

3. Landegren U. DNA diagnosis - applications and challenges. Proceedings of "*Advances in Biotechnology, an International Conference*," Swedish Council for Forestry and Agricultural Research (1990)

4. Landegren U. Molecular pathology: for the pathologist and the clinician. Book review of "Molecular Diagnostics in Pathology", Eds. Fenoglio-Preiser and Willman, Willliams and Wilkins. *Genetic Analysis Techniques and Applications)*, **8**: 246-247 (1991)

5. Landegren U. DNA probers roll för utveckling av diagnostika. Proceedings of *Laboratory Diagnostics in the 90ies* Sollentuna, Sweden (1990)

6. Landegren U. DNA diagnostik på väg in i sjukvården. *Läkartidningen* **88**: 2300-2303 (1991)

7. Landegren U. Detection of characterized mutations using the oligonucleotide ligation assay. Proceedings from *the Harden Conference on Mutation Detection in Man*, Oxford, Great Britain (1991)

8. Landegren U. Nucleic acid amplification techniques in DNA diagnostics. An analysis prepared for KABI Pharmacia Diagnostica (1991)

9. Pettersson U and Landegren U. Medicinsk genetik inför nästa sekelskifte. *Finska Läkaresällskapets Tidning* **4**:342-346 (1991)

10. Landegren U. På väg att veta allt om människans arvsmassa. GENTEKNIK - 90-TALETS TEKNIK inom medicin, jordbruk och miljö. Presentation by the Recombinant DNA Delegationen before the Swedish parliament. Stockholm, December (1992)

11. Landegren U. PCR-assisted methods in DNA analysis and molecular diagnosis. Syllabus from an advanced course, June (1992)

12. Holmström S, Boman J, Grankvist O, Landegren U, Levi R, Markė L-Å, and Olerup O. Gendiagnostik med PCR. SBU, The Swedish Board for the Evaluation

of Medical Technology, Norstedts Tryckeri AB, Stockholm ASBN 91-87890-20-8 (1993)

13. Landegren U. Ligase-mediated gene detection: theory of the method. Proceedings from the meeting "Mutations in the Human Genome", Orta San Giulio, Italy (1993)
14. Landegren U Bör vi läsa boken om människan? *Vår Lösen* **5/6**:406-411 (1993)
15. Landegren U. Nobelpris till framgångsrik kedjereaktion. *Kemisk Tidning* **14**: 24-26 (1993)
16. Landegren U. In vitro genetik Nobelprisbelönas. *Elementa* **1**: 3-6 (1994)
17. Gustavson KH and Landegren U. Genetisk diagnostik inför år 2000. Report before Swedish health care officials (1994)
18. Landegren U. Review of: The uses of life. A history of biotechnology, by Robert Bud. *Lychnos* (1994)
19. Landegren M. Genetisk analys: Människans arvsmassa på undersökningsbritsen. Report before Swedish health care officials (1996)
20. Landegren U. Hereditet: 0? Människans arvsmassa på undersökningsbritsen. *Läkartidningen* **93**: 2495-2498 (1996)
21. Landegren U. Hereditet: 0? Människans arvsmassa på undersökningsbritsen. *Nordisk Medicin* **8**: 279-283 (1996)
22. Syvänen A-C, Landegren U, Isaksson A, Gyllensten U, Brookes A. Enthusiasm mixed with skepticism about single-nucleotide polymorphism markers for dissecting complex disorders. *European Journal of Human Genetics* **7**: 98-101 (1999)
23. Landegren U. Skördetid för genomforskningen. *Läkartidningen* **96**: 3426-3428 (1999)
24. Landegren U. High-throughput genetic data capture. *Pharmaventures* (1999)
25. Dianzani I, Camaschella C, Alberto Ponzone, Piazza A, Cotton RGH, Landegren U. Spell-checking our genes. *European Journal of Human Genetics* **7**: 941-943 (1999)
26. Irma Dianzani, Ulf Landegren, Clara Camaschella, Alberto Ponzone, Alberto Piazza, Richard G.H. Cotton. Fifth international mutation detection workshop. *Human Mutation* **14**: 451-453 (1999)
27. Isaksson A, Landegren U, Syvänen A-C, Bork P, Stein C, Ortigao F, Brookes A. Discovery, scoring and utilization of human single nucleotide polymorphisms; a multidisciplinary problem. *European Journal of Human Genetics* **8**:154-156 (2000)
28. Landegren U, Antson D-O, Banér J, Barbany G, Fredriksson S, GullbergM, Isaksson A, Kwiatkowski M, Mendel-Hartvig M, Nilsson M, Olsson C. Ligase-mediated detection of genes, transcripts, and proteins. Proceedings of *The First Euroconference on Quantitative Molecular Cytogenetics, Bari* (2000)
29. Isaksson A, Landegren U, Syvänen A-C, Bork P, Stein C, Ortigao F, Brookes A. [Report from the 2nd international SNP and complex disease-meeting]. *Human Mutation* (2000)
30. Landegren U. Gendiagnostik i klinisk rutin och i biobanker. Utredning om Ethics in biomedicine. Ed. Mats G Hansson. (2002)
31. Landegren U, Genetic technology and biobanks explain disease mechanisms. From a symposium at the Swedish Medical Convention 2000. "Genetisk populations screening: " Editor The-Hung Bui T.H,. *Läkartidningen* **17**: 1920-1921 (2002)

32. Landegren U, Svensk vinkel på biotekniken (Swedish perspective on biotechnology). *Kemivärlden Biotech* Nr 4-6 (2002).

33. Landegren U. Molekylär Eskulapism. Column for *Esqulap*, No. **2**: 34 (2002)

34. Dahl N, Landegren U Eds. "Gendiagnostik i sjukvården" (Gene diagnostics in healthcare). Vetenskapsrådet ISBN 91-7307-024-6 (2003)

35. Landegren U. Varför har gendiagnostik kommit i fokus? In "Gendiagnostik i sjukvården" (Gene diagnostics in healthcare). Dahl N, Landegren U Eds Vetenskapsrådet ISBN 91-7307-024-6, p7-10 (2003)

36. Landegren U. Grundkurs i genetik. In "Gendiagnostik i sjukvården" (Gene diagnostics in healthcare). Dahl N, Landegren U Eds. Vetenskapsrådet ISBN 91-7307-024-6, p13-18 (2003)

37. Pettersson U, Nilsson K, Landegren U, Lindahl S, Lundgren E, Möller E, Nordenskjöld M. Framtida möjligheter med gendiagnostik. In "Gendiagnostik i sjukvården" (Gene diagnostics in healthcare). Dahl N, Landegren U Eds Vetenskapsrådet ISBN 91-7307-024-6, p97-104 (2003)

38. Landegren U. Cell- och molekylärbiologi. In "Medicinsk forskning för hälsa, god sjukvård och ekonomisk tillväxt". Vetenskapsrådet ISBN 91-7307-037-8, 103-106 (2003)

PATENTS

1. Landegren U and Hood L. Method of detecting a nucleotide change in nucleic acids. US Patent No 4 988 617. "OLA"

2 Landegren U. A method of processing nucleic acid samples. WO9411529, PCT/SE93/00929; US Patent No. 5 618 701. "Manifold1"

3. Kramer FR, Landegren U, Lizardi PM, Szostak J, and Tyagi S. Sensitive nucleic acid sandwich hybridization assays and kits. WO9416105, US Patent No 5 759 773. "Qbeta1"

4. Blok H, Kramer FR, Landegren U, Lizardi PM, and Tyagi S. Diagnostic assays and kits for RNA using RNA binary probes and a ribozyme ligase. WO9416103, US 5 652 107. "Qbeta2"

5. Landegren U and Kwiatkowski M. Method and reagent for detecting specific nucleotide sequences. WO 9522623. Circularizing nucleic acid probe able to interlock with a target sequence through catenation. US Patent No. 5 871 921. "Padlock 1"

6. Landegren U and Kwiatkowski M. Nucleic acid detecting reagent. WO9522623, WO9741254, US Pat 6,235,472 "Padlock2"

7 Asp A, Landegren U, and Castenius P. Method of nucelic acid transfer. WO9530773, PCT/SE95/00492,1994-05-06, US Patent No. 5 759 784. "Manifold2"

8. Burdick B, Landegren U, Sevigny P, and Ramanujam R. Method and preparation for sequential delivery of wax-embedded, inactivated biological and chemical reagents. US Patent No. 5 599 660. "Manifold3"

9. Landegren U and Sundvall M. Method of preparing oligonucleotide probes or primers, a vector therefor, and use there of. WO 9616724 US Pat 5,952,201. "Chemoenzymatic synthesis" Discontinued

10. Landegren U, Öhman O, Mendel Hartvig M, and Khorlin A. Multifunctional surfaces. WO 9617246, US pat 6,140,135. "Salamis"

11. Landegren U, Lagerkvist A. Method for detecting DNA sequence variations. WO 9623903 "Unknown mutations1"

12. Landegren U. Ultrasensitive immunoassays. WO9700446, Applied for, 1995 "Proximity1". US Pat issued

13. Landegren U and Lagerkvist A. Detection of mismatches by resolvase cleavage on a solid support. WO 9635809. US Patent No. 5 876 941. "Unknown mutations2"

14. Landegren U. Improved probing of specific nucleic acids. WO 9741254. "Padlock2"

15. Landegren U. Novel use of padlock probes. WO9709069, Applied for, 1995. "Padlock3"

16. Kwiatkowski M, Nilsson M, Landegren U. Improved support for solid phase synthesis. WO 9808857, US Pat 6,291,669. "Full-length oligonucleotides"

17. Kwiatkowski M, Landegren U, Nilsson M. *In situ* synthesis of oligonucleotides of inverse orientation. WO 9851698. "Molecular inversion"

18. Landegren U. Rolling circle replication of padlock probes. WO0161037, Applied for, 1998. "Padlock4"

19. Landegren U, Fredriksson S. Methods and kits for proximity probing. WO0161037, Applied for 2000. "Proximity2"

20. Nilsson M, Landegren U. Nucleic acid detection medium. WO0177383, Applied for 2000. "Padlock5"

21. Landegren U, Nilsson M, Gullberg M. Nucleic acid amplification method. WO03012119, Applied for 2001. "Padlock6"

22. Gullberg M, Landegren U. Nucleic acid enrichment. WO03044229, Applied for 2001. "Padlock7"